Journal of Advanced Pharmaceutical Technology & Research

LEAVES EXTRACT OF *MURRAYA KOENIGII* LINN FOR ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY IN ANIMAL MODELS

Shailly Gupta*[1], Mathew George[1], Manmohan Singhal[1], Ganesh N. Sharma[1], Vikas Garg[2]

1. School of Pharmaceutical Sciences, Jaipur National University, Jaipur (India)
2. Institute of Pharmacy, Nirma University, Ahmedabad (India)

Corresponding Author's E-mail: shailly10feb@gmail.com

Received: 19th January 2010 Revised: 18th February 2010 Accepted: 18th March 2010

Abstract

This work has been done for the investigation of the anti-inflammatory and analgesic activity of methanol extract of dried leaves of *Murraya koenigii* Linn by oral administration at dose of 100, 200 and 400 mg/kg body weight, to healthy animals. Extract was studied for its anti-inflammatory activity by using carrageenan-induced hind paw edema in albino rats and the mean increase in paw volume and % inhibition in paw volume were measured plethysmometrically at different time intervals after carrageenan (1% w/v) injection. Extract was also evaluated for analgesic activity using Eddy's hot plate method and formalin induced paw licking method in albino rats. The methanol extract showed significant ($P < 0.001$) reduction in the carrageenan-induced paw edema and analgesic activity evidenced by increase in the reaction time by eddy's hot plate method and percentage increase in pain in formalin test. The methanol extract showed anti-inflammatory and analgesic effect in dose dependent manner when compared with the control and standard drug, diclofenac sodium (10mg/kg, p.o). These inhibitions were statistically significant ($P < 0.05$). Thus our investigation suggests a potential benefit of *Murraya koenigii* in treating conditions associated with inflammatory pain.

Keywords: *Murraya koenigii*, Analgesic, anti-inflammatory, paw licking.

Introduction

Murraya koenigii is known as '*curry patta*' in hindi and widely used as spice and condiment in India and other tropical countries. It belongs to the family Rutaceae [1]. Traditionally, the plant is used as a stimulant, stomachic, febrifuge, analgesic and for the treatment of diarrhoea, dysentery; insect bites and also used to allay heat of body [2]. Previous phytochemical investigations on this plant revealed the occurrence of carbazole alkaloids [3-6]. Anti-oxidant, anti-tumour, anti-microbial, anti-inflammatory, anti-trypanocidal and mosquitocidal activities have been indicated for some of these alkaloids [7-11]. This study, therefore, intends to investigate the analgesic and anti-inflammatory activities of

the leaves of *Murraya koenigii* by studying the effects of methanol extracts of the plant on nociception induced by formalin and hot-plate, and on carrageenan induced inflammation in experimental animal models, in order to validate the folk medicinal use of this plant.

Material and Methods

Plant material

The leaves of *Murraya koenigii* were collected from the University of Rajasthan, Jaipur (Rajasthan, India) in month of March 2009. The identity of the collected plant was confirmed by P.J. Parmar, Joint Director in Botanical Survey of India (BSI), Jodhpur (Rajasthan, India). The Herbarium of the plant was deposited in the BSI against voucher specimen no. JNU/JPR/PC/SG-1

Preparation of the methanol extract

The plant material was dried under shade and powdered mechanically. The 50 gm of powder sample was defatted with petroleum ether (60-80^{0}c), and then extracted with methanol by using soxhlet apparatus. The extraction was continued till a few drops of the last portion of the extract left no residue on drying. The solvent was removed by concentrated *in vacuo* in a rotary evaporator and dried under reduced pressure. The yield of the methanol extract was 9.4%. The dried extract was stored in refrigerator until further studies.

Drugs

Diclofenac sodium, lambda Carrageenan (Sigma Chemical Co.), Formalin (Merck Specialities, Mumbai), Sodium chloride (Merck Specialities, Mumbai).

Animals

Male albino mice (25–30 g) and male albino rats (180–200 g) were used taking into account international principles and local regulations concerning the care and use of laboratory animals [12]. The animals had free access to a standard commercial diet and water *ad libitum* and were kept in rooms maintained at 22 ± 1ºC with a 12-h light/dark cycle. The institutional animal ethics committee has approved the protocol of the study (Approval no. 001/2009/IAEC/JNU).

Carrageenan-induced edema in rats [13]

5 Groups of six animals were used. Paw swelling was induced by sub-plantar injection of 0.1 ml 1% sterile lambda carrageenan in saline into the right hind paw. The methanol extracts of *M. koenigii* at dose of 100, 200 and 400 mg/kg were administered orally 60 min before

carrageenan injection. Diclofenac sodium (10 mg/kg) was used as reference drug. Control group received the vehicle only (10 ml/kg). The inflammation was quantified by measuring the volume displaced by the paw, using a plethysmometer (Ugo Basile) at time 0, 1, 2, 3, and 4 h after carrageenan injection. The difference between the left and the right paw volumes (indicating the degree of inflammation) was determined and the percent inhibition of edema was calculated in comparison to the control animals.

Formalin test [14]

The procedure was similar to that described previously by Hunskaar and Hole, 1987 and consisted of the injection of 20 µl of 2.5% solution of formalin (0.92% formaldehyde) made up in phosphate buffer (pH 7.3) in the dorsal surface of the left hind paw of the mice. Immediately, the animals were placed individually in an observation chamber made of acrylic transparent; beneath the floor, a mirror was mounted at a 45º angle to allow clear observation of the paws of the animals. The amount of time that the animal spent licking the injected paw, considered as indicative of pain, was recorded during 30 min following formalin injection. The initial nociceptive scores normally peaked 5 min after formalin injection (early phase) and 15–30 min after formalin injection (late phase), representing both the neurogenic and inflammatory pain responses, respectively [14]. Animals were treated with the methanol extract of *M. koenigii* (at dose of 100, 200 and 500 mg/kg p.o.) 1 h before the formalin injection. Control animals received only the vehicle used to dilute the substances (NaCl solution 10 ml/kg).

Hot-plate test [15]

The hot-plate was used to measure response latencies according to the method described by Eddy and Leimbach, 1953. The mice were placed on an Ugo Basile hot-plate maintained at 56ºC and the time between placement of the mouse on the platform and shaking or licking of the paws or jumping was recorded as the hot-plate latency. Mice with baseline latencies higher than 10 sec were eliminated from the study. Twenty-four hours later animals were treated with the methanol extract of *M. koenigii* (at dose of 100, 200 and 500 mg/kg p.o.) or with diclofenac sodium (10 mg/kg p.o.) 60 min., before the test. Control animals received the same volume of saline solution (10 ml/kg).

Statistical analysis

The results are expressed as mean ± S.D. (n = 6). Statistical significance was determined by analysis of variance and

subsequent followed Turkey's tests. *P* values less than 0.05 were considered as indicative of significance. The analysis was performed using INSTAT statistical software.

Results

Carrageenan-induced edema in rats

The anti-inflammatory effects of the methanol extracts of *M. koenigii* on carrageenan-induced oedema in rat's hind paws are presented in Table 1. There was a gradual increase in oedema paw volume of rats in the control group. However, in the test groups, the extract showed a significant reduction in the oedema paw volume. As indicated in Fig. 1, the oral administration of methanol extracts of *M. koenigii* at doses of 100, 200 and 400 mg / kg p.o. 1 h before carrageenan exhibited a dose-related inhibition of hind paw oedema between 2 and 4 h. The inhibitory effect was highest with 400 mg / kg. Significant effects were demonstrated by the extract. Diclofenac as reference drug (10 mg/kg orally) produced a significant inhibitory effect comparable to the extract. Extract and diclofenac exhibited 30.8 ± 8.7 and 40.93 ± 7.7% inhibition of oedema formation, respectively at 4 h after carrageenan administration.

Table 1: Anti-inflammatory effect of MEMKL in carrageenan induced paw edema.

Group(s)	Mean difference in paw volume in (ml.)			
	1 h	2 h	3 h	4 h
Control	0.46 ± 0.08	0.66 ± 0.11	0.78 ± 0.14	0.73 ± 0.21
DS	0.29 ± 0.05 [a] (17.2 ± 10.5)	0.37 ± 0.06 [a] (23.2 ± 9.6)	0.41 ± 0.10 [a] (26.6 ± 7.5)	0.16 ± 0.16 [c] (40.9 ± 7.7)
me MKL -100	0.40 ± 0.13 (4.91 ± 5.5)	0.53 ± 0.17 (9.15 ± 7.3)	0.65 ± 0.21 (8.44 ± 6.0)	0.54 ± 0.13 [a, d] (12.7 ± 6.2)
meMKL -200	0.30 ± 0.12 (15.5 ± 13.9)	0.43 ± 0.18 (18.3 ± 12.5)	0.54 ± 0.22 (17.5 ± 9.7)	0.42 ± 0.11 [b] (22.8 ± 10.7)
meMKL -400	0.27 ± 0.09 [a] (20.4 ± 2.9)	0.40 ± 0.14 [a] (22.5 ± 9.6)	0.48 ± 0.16 (23.3 ± 7.7)	0.33 ± 0.08 [c] (30.8 ± 8.7)

Values are mean ± S.D. (n=6); values in bracket indicate % inhibition of oedema. a=P<0.05 when compared to control group. b=P<0.001 when compared to control group. c=P<0.0001 when compared to control group.

d=P<0.01when compared to standard diclofenac sodium (DS) treated group

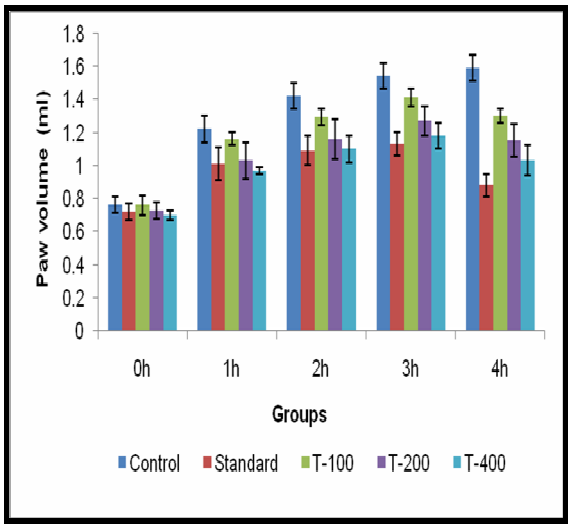

Fig. 1 Anti-inflammatory effect of MEMKL in carrageenan induced rat paw edema

Formalin test

Intraplantar injection of 2.5% formalin evoked a characteristic biphasic licking response. The duration of licking for the early phase (0–5 min) was 49.66 ± 1.36 sec and for the late phase (15–30 min) was 83 ± 1.26 sec in control groups. As shown in Table 2 and Fig. 2, pretreatment (60 min) with different doses of methanol extract of *M. koenigii* had significant effect against the duration of licking activity in the early as well as late phase, doses of 100, 200 and 400 mg/kg p.o. produced a marked reduction of the licking time in the early phase (35.61 ± 6.01, 64.69 ± 3.82 and 75.77 ± 4.07%) and late phase (69.85 ± 5.09, 84.33 ± 1.13 and 87.94 ± 1.74%), respectively.

Table 2: Effect of methanol extract of MEMKL in the formalin test in mice

Group(s)	Numbers of paw lickings		% increase in latency time	
	Phase-I	Phase-II	Phase-I	Phase-II
Control	49.66 ± 1.36	83 ± 1.26	-	-
DS	22.01 ± 1.6[a]	18 ± 0.89[a]	55.70 ± 3.17	78.30 ± 1.22
T-100	32.01 ± 3.4[a]	25 ± 4.09[a]	35.61 ± 6.01	69.85 ± 5.09
T-200	17.50 ± 1.5[a,b]	13 ± 0.89[a,b]	64.69 ± 3.82[b]	84.33 ± 1.13[b]
T-400	12.00 ± 1.7[a,b]	10 ± 1.41[a,b]	75.77 ± 4.07[b]	87.94 ± 1.74[b]

Values are mean ± SD (n=6) of licking time in the Phase-I and Phase-II.

a= P<0.001 when compared to control group.

b= P<0.001 when compared to standard diclofenac sodium (DS) treated group.

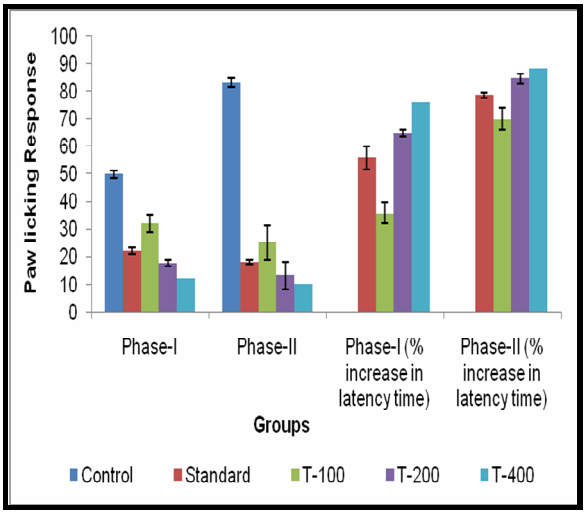

Fig. 2: Analgesic effect of methanol extract of MEMKL in the formalin test in mice

Table 3: Analgesic effect of methanol extract of MEMKL in the hot plate test

Group(s)	Pain latency time (Sec.)			
	0 min	30 min	60 min	120 min
Control	0.97 ± 0.03	1.0 ± 0.02	1.1 ± 0.02	1.0 ± 0.05
DS	1.07 ± 0.04	1.86 ± 0.02[a] (9.34± 4.81)	2.87 ± 0.02[a] (46.05 ± 1.22)	4.79 ± 0.16[a] (61.67 ± 0.81)
T-100	1.06 ± 0.04	1.6 ± 0.06[a] (8.49 ± 6.57)	1.91 ± 0.06[a] (37.29 ± 3.23)	3.38 ± 0.09[a] (42.40 ± 2.70)
T-200	1.11 ± 0.02	1.83 ± 0.02[a] (12.61 ± 4.32)	2.46 ± 0.06[a] (45.17 ± 1.39)	4.73 ± 0.12[a] (55.34 ± 1.14)
T-400	1.13 ± 0.03	2.93 ± 0.03[a,b] (14.15 ± 3.25)	4.81 ± 0.04[a,b] (65.75 ± 0.53)	5.79 ± 0.26[a,b] (77.16 ± 0.47)

Values are mean ± SD (n=6); values in bracket indicate percentage inhibition in pain.

a= P<0.001 when compared to control group.

b= P<0.001 when compared to standard diclofenac sodium (DS) treated group.

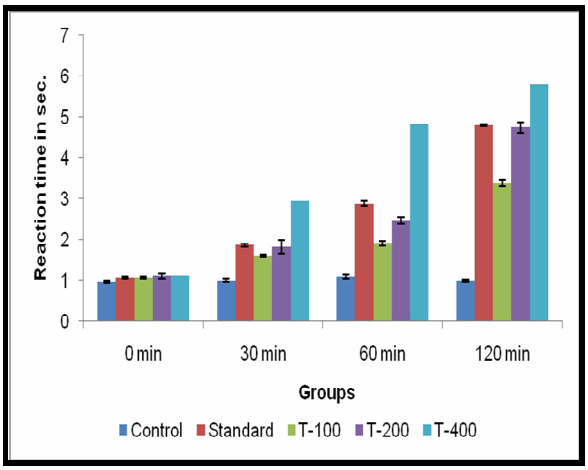

Fig. 3: Effect of methanol extract of MEMKL in the hot plate test

Hot-plate test

Diclofenac sodium at a dose of 10 mg/kg and methanol extract of *M. koenigii* produced significantly increased the pain latency, as compared to the control group. Methanol extract of *M. koenigii* at doses of 100, 200 and 400 mg/kg p.o. produced significantly percentage increased in pain (42.40 ± 2.70, 55.34 ± 1.14 and 77.16 ± 0.47) 2 h after drug administration as shown in Table 3 and Fig. 3.

Discussion

The present study provides evidence that the methanol extract of *Murraya koenigii* acts as an anti-inflammatory agent in rats in acute inflammation model. Carrageenan induced inflammation is most commonly used as an experimental model for evaluating the anti-inflammatory potency of compounds or natural products [16].

Moreover the experimental model exhibits a high degree of reproducibility [17]. Carrageenan induced paw edema is biphasic event. The first phase is attributed to the release of histamine, serotonin and kinins. The second phase of edema is due to release of prostaglandins, protease and lysosome. The second phase is sensitive to most clinically effective anti-inflammatory drugs [18]. The results of present study indicate the role of MEMKL (methanol extract of *Murraya koenigii* leaves) against carrageenan induced acute inflammation is significant. The MEMKL at a dose of 100 and 200 mg/kg suppressed only the second phase of carrageenan induced inflammation but at a dose of 400 mg/kg significantly suppress both first and second phase of carrageenan induced inflammation. The standard diclofenac sodium significantly suppresses the biphasic response of carrageenan induced inflammation. So, the anti-inflammatory effect of MEMKL at a dose of 400 mg/kg may be due to its suppression action on prostaglandin, protease or lysosome synthesis or activity.

Two different analgesic testing methods were employed in the current investigation with the objective to identifying possible peripheral and central effects of the MEMKL. Using, both hot plate test and formalin induced paw licking response in mice, it was observed that the plant extracts possessed analgesic effects against both models. The observations also indicated that the extracts have both central and peripheral effects. To evaluate for a possible central anti-nociceptive effect of the MEMKL, the hot plate test was used [19] possibly acting on a descending inhibitory pain pathway [20]. The paw-licking hot plate response is a more complex supraspinally organized behavior [21]. The μ receptor has generally been regarded as the receptor type associated with pain relief and has been shown to be potent in regulating thermal pain [22]. Activation of μ_2 opioid subtype leads to spinal analgesia and commonly through constipation adverse effect [23]. Therefore, by considering several reports, the antinociceptive activity of MEMKL is likely to be mediated centrally.

The MEMKL also possesses anti-nociceptive activity in the formalin test. The advantage of using the formalin model of nociception is that it can discriminate between central and peripheral pain components [24]. The test consists of 2 different phases. The first one (neurogenic phase) is generated in the periphery through the activation of nociceptive neurons by the

direct action of formalin, and the second phase (inflammatory phase) occurs through the activation of the ventral horn neurons at the spinal cord level. Morphine, a typical narcotic analgesic drug, can inhibit nociception in both phases [25] and NSAIDs by acting supraspinally can reduce the pain in both phases [26]. Inhibition of the late phase is due to inflammation causing a release of serotonin, histamine, bradykinin and prostaglandins, which at least to some degree can cause the sensitization of the central nociceptive neurons[27]. In the present study, MEMKL produced antinociception against both neurogenic and inflammatory phase of formalin. The effect of MEMKL was more pronounced against the inflammatory phase in the formalin test, the antinociceptive is likely to be mediated peripherally.

From the present work it can be speculated that analgesic effect of MEMKL may be linked to processes involved in the prevention of sensitization of nociceptors, down regulation of the sensitized nociceptors and/or blockade of the nociceptors at peripheral and/or central levels [28].

Acknowledgements

Authors thank Joint Director of Botanical Survey of India, Jodhpur (Rajasthan) for the authentication of specimens of *Murraya koenigii*.

References

1. Satyavati G. V., Gupta A. K. & Tandon N. *Medicinal Plants of India*. vol. 2. New Delhi. Indian Council of Medical Research, India. 1987: 289-299.
2. Kirtikar K. R., Basu, B. D. Indian Medicinal Plants. Vol 1, 2nd edition. Bishen Singh Mahendra Pal Sigh, India. 1993: 472-474.
3. Narasimhan N. S., Paradhar M. V. & Chitguppi V. P., Structures of mahanimbine and koenimbine. *Tetrahedron Lett*. 1968; 53: 5501– 5504.
4. Chowdhury B. K. & Chakraborty D. P. Mukeic acid, the first carbazole carboxylic acid from a plant source. *Phytochemistry*. 1971; 10: 1967–1970.
5. Chakraborty D. P., Roy S. & Ruha R. Structure of mukonidine, *J. Indian Chem. Soc*. 1978; 55: 1114–1115.
6. Rao R. A. V., Rhide K. S. & Mujumdar R. B.. Mahanimbinol from *Murraya koenigii*. Chem. *Ind.* 1980; 17: 697–698.
7. Das K. C., Chakraborty D. P. & Bose P. K. Antifungal activity of some constituents of *Murraya koenigii* Spreng. *Experentia.* 1965; 21: 340.
8. Chakrabarty M., Nath A. C., Khasnobis S., Chakrabarty M., Konda Y., Harigaya

Y. & Komiyama K.. Carbazole alkaloids from *Murraya koenigii*. *Phytochemistry*. 1997; 46: 751–755.

9. Nutan M. T. H., Hasnat A. & Rashid M. A., Antibacterial and cytotoxic activities of *Murraya koenigii*. *Fitoterapia.* 1998; 69: 173–175.
10. Itoigawa M., Kashiwada Y., Ito C., Furukawa H., Tachibana Y., Bastow K. F. & Lee K. H., Antitumour agents Carbazole alkaloid murrayaquinone-A and related synthetic carbazolequinones as cytotoxic agents. *J. Nat. Prod*. 2000; 63: 893–897.
11. Nakatani N. Phenolic antioxidants from herbs and spices. vol 13. 14th edition. Biofactors. 2000: 141–146.
12. Olfert E. D., Cross B. M., McWilliam A. A. Canadian Council of Animal Care guide to the care and use of experimental animals. vol. 1. 2nd edition. 1993.
13. Winter C. A., Risley E. A. & Nuss G. W., Carrageenan-induced edema in hind paws of the rat as an assay for antiinflammatory drugs. *Proc Soc Exp Biol Med*. 1962; 111: 544–52.
14. Hunskaar S. & Hole K., The formalin test in mice: dissociation between inflammatory and non inflammatory pain. *Pain*. 1987; 30: 103–104.
15. Eddy N. B. & Leimbach D. Synthetic analgesics. II. Dithienylbutenyl and dithienylbutilamines. *J Pharmacol Exp Ther*. 1953; 107: 385–393.
16. Banerjee S., Sur T. P., Mandal S., Das P. C. & Sikdar S. Assessment of the anti-inflammatory effects of *Swertia chirata* in acute and chronic experimental models in male albino rats. *Indian J Pharmacol*. 2000; 32: 21-24.
17. Kale M., Misar A. V., Dave V. & Joshi M. M. Anti-inflammatory activity of *Dalbergia lanceolaria* barks ethanol extract in mice and rats. *Journal of Ethnopharmacology*. 2007; 112: 300-304.
18. Vinegar R., Schreiber W. & Hugo R. Biphasic development of carrageenan edema in rats. *Journal of Pharmacology Experiment and Therapeutics*. 1969: 96-103.
19. Marchioro M., Blank Mde, F., Mourao R. H. & Antoniolli A. R. Antinociceptive activity of aqueous extract of *Erythrina velutina* leaves. *Fitoterapia*. 2005; 76: 637–642.
20. Richardson J. D., Aanonsen L. & Hargreaves K. M., Antihyperalgesic effects of spinal cannabinoids. *European Journal of Pharmacology*. 1998; 345: 145–153.

21. Chapman C. R., Casey K. L., Dubner R., Foley K. M., Gracely R. H. & Reading A. E. Pain measurement: an overview. *Pain.* 1985*;* 22: 1–31.
22. Dhawan B. N., Cesselin F., Raghubir R., Reisine T., Bradley P. B., Protoghese P. S. & Haman M. Classification of opioid receptors. *Pharmacological Reviews.* 1996; 48: 567–592.
23. Lipman A. G. & Jackson R. C. Principles and Practice of Pain Medicine. 2nd edition. New York: McGraw-Hill; 2004: 585–588.
24. Tjolsen A., Berge O. G., Hunskaar S., Rosland J. H. & Hole K. The formalin test: an evaluation of the method. *Pain*. 1992; 51: 5–17.
25. Shibata M., Ohkubo T., Takahashi H. & Inoki R., Modified formalin test: characteristic biphasic pain response. *Pain.* 1989; 38: 347–352.
26. Martindale J., Bland-Ward P. A. & Chessell I. P. Inhibition of C-fibre mediated sensory transmission in the rat following intraplantar formalin. *Neuroscience Letters*. 2001; 316: 33–36.
27. Verma P. R., Joharapurkar A. A., Chatpalliwar V. A., Asnani A. Antinociceptive activity of alcoholic extract of *Hemidesmus indicus* R.Br. in mice, *Journal of Ethnopharmacology*. 2005; 102: 298–301.
28. Ferreira S. H. In: Sandier M. and Collins M., editor. A classification of peripheral analgesics based upon their mode of action, Migraine a spectrum of ideas. New York: Oxford Medical Publications; 1990.